

Claims

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1. A fluorescent protein derived from Green Fluorescent Protein (GFP) or any functional analogue thereof, wherein the amino acid in position 1 preceding the chromophore has been mutated to provide an increase in fluorescence intensity.
2. A fluorescent protein according to claim 1, wherein the chromophore is in position 65-67 of the predicted primary amino acid sequence of GFP.
- 10 3. A fluorescent protein according to claim 1 resulting in an increased fluorescence in cells expressing said fluorescent protein when said cells are incubated at a temperature of 30°C or above 30°C, preferably at a temperature of from 32°C to 39°C, more preferably at a temperature of from 35°C to 38°C, and most preferably at a temperature of about 37°C.
- 15 4. A fluorescent protein according to claim 1, said protein being derived from *Aequorea victoria* or *Renilla reniformis*.
- 20 5. A fluorescent protein according to claim 1, wherein the amino acid F in position 64 of GFP or Y66H-GFP has been substituted by an amino acid selected from the group consisting of L, I, V, A and G.
- 25 6. A fluorescent protein according to claim 1, wherein the amino acid F in position 1 preceding the chromophore has been substituted by L and the amino acids of the chromophore include SYG, SHG or TYG.
7. A fluorescent protein according to claim 1 and having the amino acid sequence of Fig. 3, Fig. 4 or Fig. 5 herein.
- 30 8. A fusion compound consisting of a fluorescent protein (GFP) according to claim 1, wherein said GFP is linked to a polypeptide.
- 35 9. A fusion compound according to claim 8 wherein the polypeptide is a kinase, preferably the catalytic subunit of protein kinase A, or protein kinase C, or Erk1, or a cytoskeletal element.
- 40 10. A nucleotide sequence coding for the Fluorescent Protein of claim 1.
11. A nucleotide sequence according to claim 10 selected from the sequences shown in Fig. 3, Fig. 4 and Fig. 5.
- 45 12. A DNA construct comprising a suitable control region or regions and a nucleotide sequence according to claim 10, the sequence being under the control of the control region.
13. A DNA construct according to claim 12 being under the control of the native GFP promoter, or a mammal constitutive or regulatory promoter, a viral promoter, a yeast promoter, a filamentous fungi promoter, or a bacterial promoter.

14. A host transformed with a DNA construct according to claim 12.

15. A host according to claim 14 selected from the following: organisms and cells belonging to bacteria, yeast, fungi, protozoans and higher eucaryots .

16. A process for preparing a polypeptide, comprising cultivating a host according to claim 14 and obtaining therefrom the polypeptide expressed by said nucleotide sequence.

17. A process according to claim 16 wherein the expression of the nucleotide sequence is effected by the native GFP promoter.

18. Use of the Fluorescent Protein of claim 1, ~~2, 3, 4, 5, 6 or 7~~ in an *in vitro* assay for measuring protein kinase activity, or dephosphorylation activity, wherein said fluorescent protein in purified form is added to a biological sample, preferably a cell extract, and any change in fluorescence is recorded.

19. Use of the host of claim 14 ~~or 15~~ in an *in vivo* assay for measuring metabolic activity, preferably kinase activity and dephosphorylating activity.

20. Use of the fluorescent protein of claim 1, ~~2, 3, 4, 5, 6 or 7~~ as a reporter for gene expression in living cells.

21. Use of the fluorescent protein of claim 1, ~~2, 3, 4, 5, 6 or 7~~ for the simultaneous monitoring of more than one gene in living, intact cells.

22. Use of two or more of the fluorescent protein of claim 1, ~~2, 3, 4, 5, 6 or 7~~ as organelle or cell tags for simultaneous visualisation of organelle or cell processes in living cells.